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## First mitogenome for the subfamily Miltogramminae (Diptera: Sarcophagidae) and its phylogenetic implications

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**Key words.** Diptera, Calyptratae, Sarcophagidae, Miltogramminae, mitogenome, flesh fly, phylogeny

**Abstract.** The mitochondrial genome of *Mesomelena mesomelaena* (Loew, 1848) is the first to be sequenced in the flesh fly subfamily Miltogramminae (Diptera: Sarcophagidae). The 14,559 bp mitogenome contains 37 typical metazoan mitochondrial genes: 13 protein-coding genes, two ribosomal RNA genes and 22 transfer RNA genes, with the same locations as in the insect ground plan. All the protein-coding genes have the start codon ATN, except for *cox1* (TCG). Eight protein-coding genes have the stop codon TAA, while the remaining five have the stop codon T (*cox1*, *cox2*, *nad5*, and *nad4*) or TAG (*cytb*). Synonymous and non-synonymous substitution rates (Ks and Ka) for each protein-coding gene indicate that these genes evolved primarily under negative (or purifying) selection (Ka < Ks). Phylogeny of Sarcophagidae is proposed based on all the sarcophagid mitogenomes in GenBank, and the subfamily topology is reconstructed as (Sarcophaginae (Paramacronychiinae, Miltogramminae)).

### INTRODUCTION

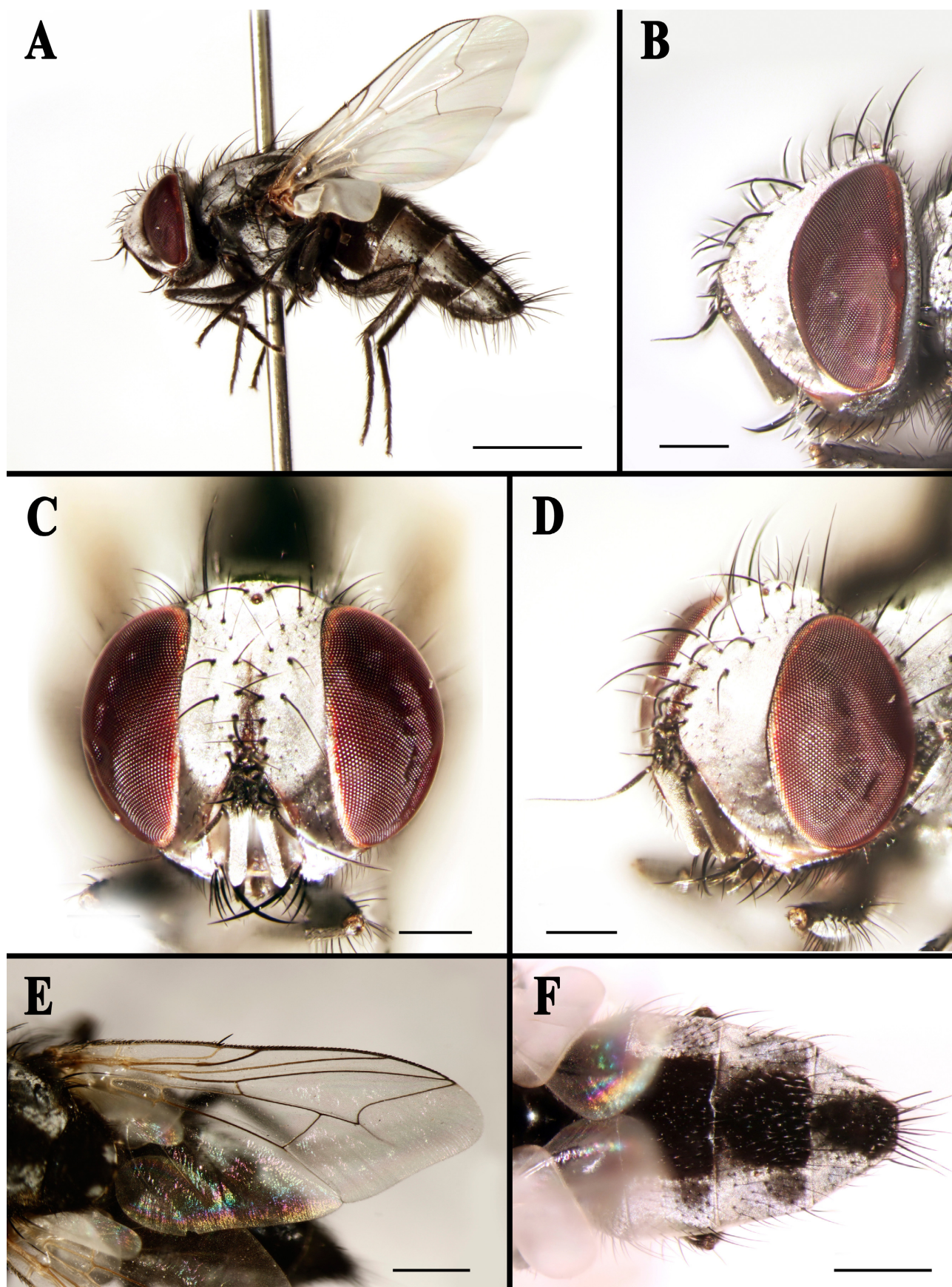
With ~3000 described species worldwide (Pape et al., 2011), the Sarcophagidae (Diptera: Calyptratae), also known as flesh flies, are generally subdivided into three subfamilies, Miltogramminae, Paramacronychiinae and Sarcophaginae (Pape, 1996; Piwczynski et al., 2017). It is a young lineage within the schizophoran super-radiation of Diptera (Wiegmann et al., 2011; Cerretti et al., 2017) with diverse life habits, e.g. kleptoparasitism of Hymenoptera (Spofford et al., 1989), predators of other insects, spider egg sacs and reptile eggs (Pickens, 1981; Lopes, 1982; Mullen et al., 1984; Trauth & Mullen, 1990; Pape, 1996), parasitoids of insects and snails (Pape, 1994; McKillup et al., 2000; Stucky, 2015) and necro/coprophyagy (Bänziger & Pape, 2004). Thus, the Sarcophagidae are of important ecological, medical and forensic importance. Mitochondrial genomes (mitogenomes) are thought to be reliable markers for phylogeny reconstruction and taxonomic diagnosis (Nelson et al., 2012; Cameron, 2014). Here we document the nearly-complete mitogenome of *Mesomelena mesomelaena* (Loew, 1848) (Figs 1–2), which is the first nearly-complete mitogenomic data for the Miltogramminae and we reconstruct the phylogeny of the Sarcophagidae based on all the mitogenomes in GenBank.

### MATERIALS AND METHODS

Two dry specimens of *M. mesomelaena*, whole body of one specimen (collected in 2014), thorax of another (collected in 2015, voucher BFU-06574), were used for DNA extraction using a QIAGEN DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany). The mitogenome was amplified in overlapping fragments by 18 primer pairs (Weigl et al., 2010; Zhang et al., 2013) and a pair of specific primers designed from sequenced fragments (Table 1). The PCR was performed in a reaction volume of 25 µL, which contained 1 µL of genome DNA, 1 µL of each primer (10 µM), 2.5 µL of 10 × Es Taq PCR Buffer (Beijing Cowin Bioscience Co., Ltd., China), 2 µL of dNTPs mixture (2.5 mM each) (Beijing Cowin Bioscience Co., Ltd., China), 0.3 µL Es Taq DNA Polymerase (1.5 U) (Beijing Cowin Bioscience Co., Ltd., China) and 17.2 µL sterling double distilled water. The PCR cycles for all amplifications were the same as used by Zhang et al. (2016b). Products were resolved in 1% agarose gels and stained with Gold View (Beijing Cool Technology Co., Ltd., China), then purified and sequenced bidirectionally by BGI Sequencing (Beijing, China). After assembly of raw sequences by SeqMan (DNASTar, Steve ShearDown, 1998–2001 version DNASTAR Inc., USA), the annotation of the mitogenome was performed as described in Zhang et al. (2016b). The mitogenome map was produced using CGView (Grant & Stothard, 2008).

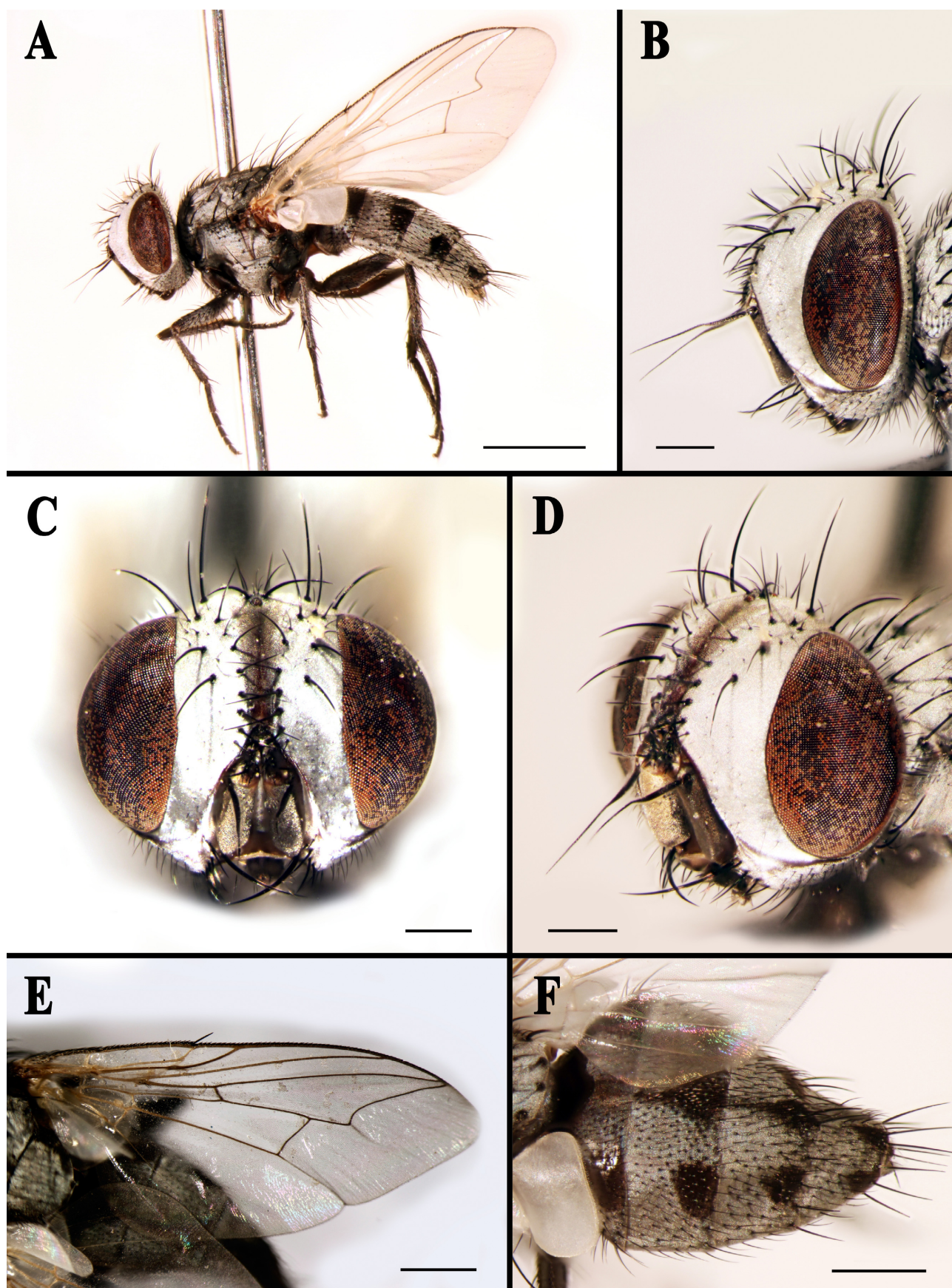
All the mitochondrial genomes of Sarcophagidae in GenBank were included in the present study, except for two sequences: one with locus KP861920 [stated to be *Sarcophaga crassipalpis*],

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**Fig. 1.** *Mesomelena mesomelaena* (Loew, 1848), male. A – habitus, lateral view; B – head, lateral view; C – head, anterior view; D – head, anterolateral view; E – right wing, dorsal view; F – abdomen, dorsal view. Scales: A – 2 mm, B–D – 0.5 mm, E and F – 0.5 mm.





**Fig. 2.** *Mesomelena mesomelaena* (Loew, 1848), female. A – habitus, lateral view; B – head, lateral view; C – head, anterior view; D – head, anterolateral view; E – right wing, dorsal view; F – abdomen, dorsal view. Scales: A – 2 mm, B–D – 0.5 mm, E and F – 0.5 mm.

**Table 1.** Primer pairs used to amplify the mitogenome of *Mesomelana mesomelaena* (Loew, 1848).

Primers	Sequence (5'–3')	Size (bp)	Reference
0006_F/R	TGAATTGCCTGATAAAAGG CTCCAATTAAGCTCCTGGATG	~1500	Weigl et al., 2010
0007_F/R	GGTGGATTACCCCATTTTATAGG TTAGCTAAAATTACTCCAGTTAATCCTCC	~1400	Weigl et al., 2010
0008_F/R	GAGGAGATCCAATTCTCTATCAACA CAATGACAATTGGTATAAAGCTGTG	~1400	Weigl et al., 2010
0009_F/R	CATTGATTTGCATTCAAAAAGTATTG GCTGCTATAGCAGCTCCTACKCC	~1200	Weigl et al., 2010
0010_F/R	CCATAAGTAGAAATATAAGGTATAAATCA TATTGATTTGTGGTATCAAGATAAG	~1450	Weigl et al., 2010
0011_F/R	CAGTAATTTTATTAAACATGAATTGGAGC GCGACCTCGATGTTGGATTAAG	~1350	Weigl et al., 2010
0013_F/R	TTCACCTCAAGATAGCTCATCTCCT GGTCATGGACTATAYTCTACTA	~1600	Weigl et al., 2010
0014_F/R	GCAGTTCGATTAAACAGCWAA AGTGATAAGCCTCTTTTGGCTTC	~1500	Weigl et al., 2010
0016_F/R	CAATTCTATTAATTAAGAAATTTCTCC CTTATTTTGTATTACAAGACCAATG	~1400	Weigl et al., 2010
0018_F/R	CCTTTACGAATYAAACACCC CTATCTTATGTTTCAAAACATATGC	~1150	Weigl et al., 2010
0019_F/R	CTAAATTTATTGCACTAATCTGCC TTGTACCTTGTGTATCAGGG	~1150	Weigl et al., 2012
0036F/R	TAGCWGCGWGGTAATCAAGA GCTCCTCCWACWTTAAAT	~930	Zhang et al., 2013
0038F/R	TCATATCAYTRACACACCA GAGGKTATCARCCWGAACG	~960	Zhang et al., 2013
0040F/R	GCHCCTTCACAWACTCTAAAWGT CRTAATAWATTCTCGTCCTA	~1100	Zhang et al., 2013
0043F/R	ATYTATAGGGTCTTCTCGTCT AATATGYACACATCGCCCGTC	~990	Zhang et al., 2013
0044F/R	GACGGGCGARTGTGCATA CCAGCAGTCGCGGTTATAC	~530	Zhang et al., 2013
0046F/R	AGGAGCWTGAATAGGWTTAGA RTGGCTGAAGTTWAGGCRATA	~1000	Zhang et al., 2013
0049F/R	ATTTTCYGTATTYGACCCYTC TCTCGWAWACATCTCGTCAT	~830	Zhang et al., 2013
0056F/R	CTTTYACAATACTAWTWMAC WAAACTAGGATTAGATACC	~600	Zhang et al., 2013
0071F/R*	ATGACTGAAAGCAAGTAT ATTAGCTGTTAATCGTAC	~750	

\* Primer pair designed using Primer Premier 5.0 (Lalitha, 2000).

which gives a mismatch with the standard *cox1* barcode region for *Sarcophaga crassipalpis* (locus: JN964810) (Meiklejohn et al., 2012) from BOLD (<http://www.barcodinglife.org/>) and one with locus KT272859, which is stated to be *Sarcophaga bullata*, but which shows 99.96% identity in protein-coding genes (PCGs) to the locus NC\_026667 (*Sarcophaga crassipalpis*).

Nineteen mitogenomes of Oestroidea (Table 2) were downloaded from GenBank to reconstruct the phylogeny of Sarcophagidae,

with *Lucilia cuprina* as an outgroup. All 15 genes were aligned separately with MAFFT v 7.3.1 (Katoh & Standley, 2016), following Kutty et al. (2014). After alignment, all 13 PCGs and two rRNAs were concatenated using SequenceMatrix v 1.7.8 (Meier et al., 2006). Subsequently, PartitionFinder v 2.1.1 (Lanfear et al., 2016) was used to evaluate the best partitioning schemes based on BIC (Bayesian Information Criterion) using the greedy option with branch lengths estimated as “unlinked”, with the dataset partitioned by genes. Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) as described in Zhang et al. (2016b).

Synonymous and non-synonymous substitution rates (Ks and Ka) for each species of each protein-coding gene (PCG) alignment were calculated using DnaSP version 5.10.1 (Librado & Rozas, 2009), with stop codons and codons with alignment gaps excluded. A plot of pairwise Ka versus Ks for each gene was then constructed using Sigmaplot v 12.5 (Systat Software Inc. California, USA).

## RESULTS AND DISCUSSION

As the control region of *M. mesomelaena* could not be amplified, the small ribosomal RNA gene is incomplete at the 5' end. The nearly-complete mitogenome of *M. mesomelaena* (14,559 bp) was submitted to GenBank (Accession Number: KY003227).

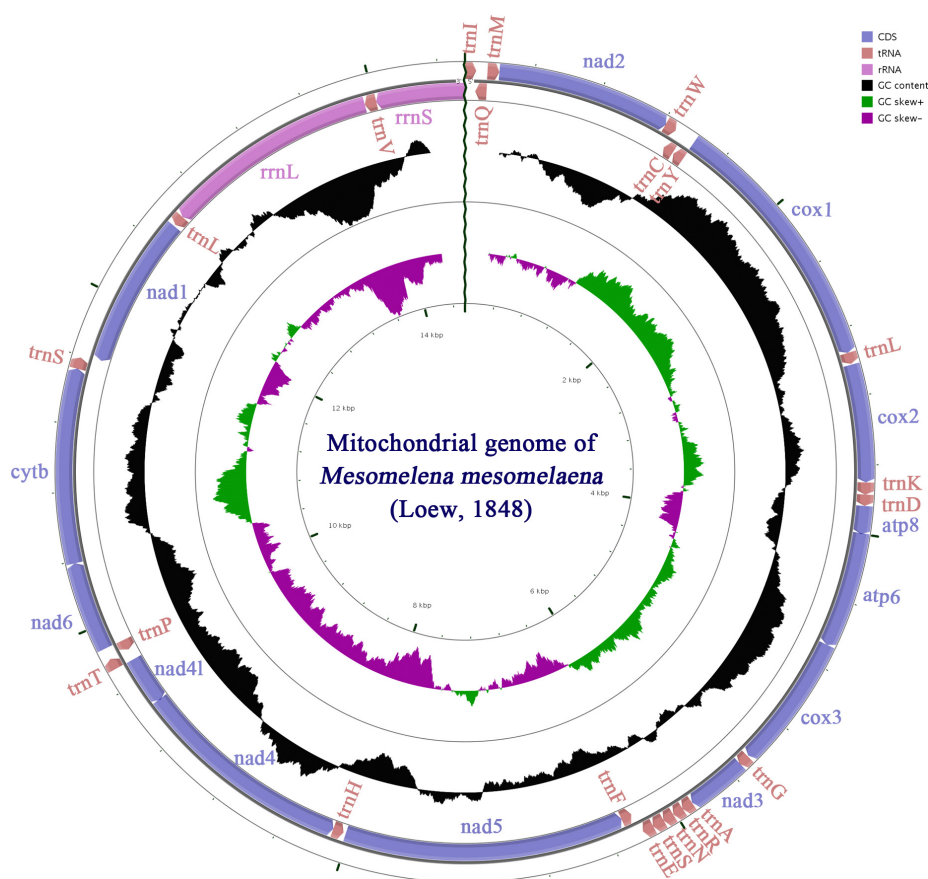
The mitogenome contains 13 PCGs, two ribosomal RNA genes (rRNA) and 22 transfer RNA genes (tRNA), with the same locations as generally in Diptera (i.e., Nelson et al., 2012; Zhang et al., 2016b) and ancestral insects (see Fig. 2 in Cameron, 2014) (Table 3, Fig. 3). The overall nucleotide composition exhibits high AT content (76.9%) similar to other calyptrates (Zhang et al., 2016b).

All PCGs share the start codon of ATT or ATG, except for *cox1* (TCG), *nad3* and *nad1* (ATA). Four genes (*cox1*, *cox2*, *nad5*, and *nad4*) use the incomplete stop codon T, and *cytb* uses TAG. The remaining eight PCGs have the typical stop codon of TAA.

The selection acting on a gene may be detected by comparing the synonymous (Ks) and non-synonymous (Ka) nucleotide distances between gene sequences (Hurst, 2002). Higher Ka than Ks indicates positive selection, and lower Ka than Ks indicates negative selection. The Ka value is obviously lower than the Ks value for all sarcophagid

**Table 2.** Taxa included in the phylogenetic analyses in this study.

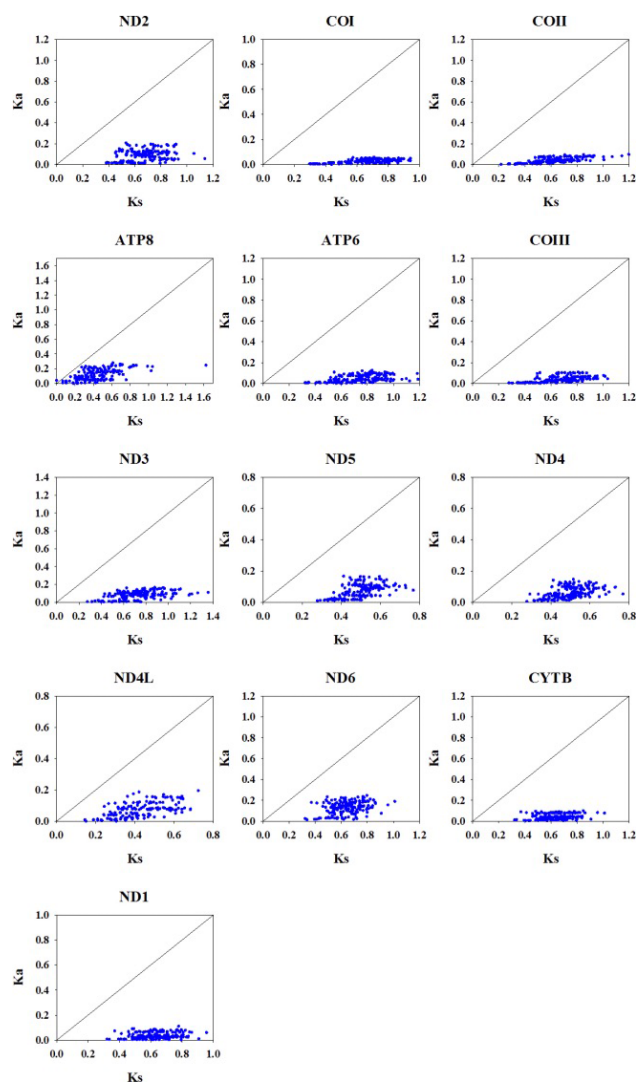
Family	Subfamily	Species	Locus	Reference
Calliphoridae	Luciliinae	<i>Lucilia cuprina</i>	NC_019573	Nelson et al., 2012
	Polleniinae	<i>Pollenia rudis</i>	JX913761	Nelson et al., 2012
Sarcophagidae	Sarcophaginae	<i>Sarcophaga melanura</i>	NC_026112	Zhang et al., 2016a
	Sarcophaginae	<i>S. portschinskyi</i>	NC_025574	Shi et al., 2016
	Sarcophaginae	<i>S. peregrina</i>	NC_023532	Zhong et al., 2016
	Sarcophaginae	<i>S. impatiens</i>	NC_017605	Nelson et al., 2012
	Sarcophaginae	<i>S. crassipalpis</i>	NC_026667	Ramakodi et al., 2015
	Sarcophaginae	<i>S. africa</i>	NC_025944	Fu et al., 2016
	Sarcophaginae	<i>S. similis</i>	NC_025573	Yan et al., 2016
	Sarcophaginae	<i>S. albiceps</i>	NC_028413	Liao et al., 2016
	Sarcophaginae	<i>Ravinia pernix</i>	NC_026196	Guo et al., 2016
	Sarcophaginae	<i>Boettcheria latisterna</i>	KT272848	Junqueira et al., 2016
	Sarcophaginae	<i>B. bisetosa</i>	KT272844	Junqueira et al., 2016
	Paramacronychiinae	<i>Wohlfahrtia magnifica</i>	KU578263	Zhang et al., 2016b
	Miltogramminae	<i>Mesomelana mesomelaena</i>	KY003227	present study
Tachinidae	Exoristinae	<i>Elodia flavipalpis</i>	NC_018118	Zhao et al., 2013
	Dexiinae	<i>Rutilla goerlingiana</i>	NC_019640	Nelson et al., 2012
Oestridae	Cuterebrinae	<i>Dermatobia hominis</i>	NC_006378	Azeredo-Espin et al., 2004
	Hypodermatinae	<i>Hypoderma lineatum</i>	NC_013932	Weigl et al., 2010



**Table 3.** Organization of the mitogenome of *Mesomelena mesomelaena* (Loew, 1848).

Gene	Product	Strand	Location	Length (bp)	Codon	
					Start/Anti	Stop
<i>trnI</i>	tRNA-Ile	N	1–65	65	GAT	
<i>trnQ</i>	tRNA-Gln	J	63–131	69	TTG	
<i>trnM</i>	tRNA-Met	N	131–199	69	CAT	
<i>nad2</i>	NADH2	N	200–1216	1017	ATT	TAA
<i>trnW</i>	tRNA-Trp	N	1215–1282	68	TCA	
<i>trnC</i>	tRNA-Cys	J	1275–1337	63	GCA	
<i>trnY</i>	tRNA-Tyr	J	1345–1410	66	GTA	
<i>cox1</i>	COX1	N	1409–2942	1534	TCG	T
<i>trnL</i>	tRNA-Leu	N	2943–3008	66	TAA	
<i>cox2</i>	COX2	N	3014–3701	688	ATG	T
<i>trnK</i>	tRNA-Lys	N	3702–3772	71	CTT	
<i>trnD</i>	tRNA-Asp	N	3772–3837	66	GTC	
<i>atp8</i>	ATP8	N	3838–4002	165	ATT	TAA
<i>atp6</i>	ATP6	N	3996–4673	678	ATG	TAA
<i>cox3</i>	COX3	N	4673–5461	789	ATG	TAA
<i>trnG</i>	tRNA-Gly	N	5467–5531	65	TCC	
<i>nad3</i>	NADH3	N	5529–5885	357	ATA	TAA
<i>trnA</i>	tRNA-Ala	N	5887–5951	65	TGC	
<i>trnR</i>	tRNA-Arg	N	5951–6013	63	TCG	
<i>trnN</i>	tRNA-Asn	N	6015–6080	66	GTT	
<i>trnS</i>	tRNA-Ser	N	6080–6149	70	GCT	
<i>trnE</i>	tRNA-Glu	N	6149–6214	66	TTC	
<i>trnF</i>	tRNA-Phe	J	6233–6298	66	GAA	
<i>nad5</i>	NADH5	J	6299–8018	1720	ATT	T
<i>trnH</i>	tRNA-His	J	8034–8099	66	GTG	
<i>nad4</i>	NADH4	J	8100–9438	1339	ATG	T
<i>nad4l</i>	NADH4L	J	9432–9728	297	ATG	TAA
<i>trnT</i>	tRNA-Thr	N	9731–9795	65	TGT	
<i>trnP</i>	tRNA-Pro	J	9796–9860	65	TGG	
<i>nad6</i>	NADH6	N	9863–10387	525	ATT	TAA
<i>cytb</i>	CYTB	N	10387–11523	1137	ATG	TAG
<i>trnS2</i>	tRNA-Ser	N	11522–11589	68	TGA	
<i>nad1</i>	NADH1	J	11606–12544	939	ATA	TAA
<i>trnL</i>	tRNA-Leu	J	12555–12619	65	TAG	
<i>rrnL</i>	16S rRNA	J	12620–13941	1322		
<i>trnV</i>	tRNA-Val	J	13942–14013	72	TAC	
<i>rrnS</i>	12S rRNA	J	14013–14559	547		

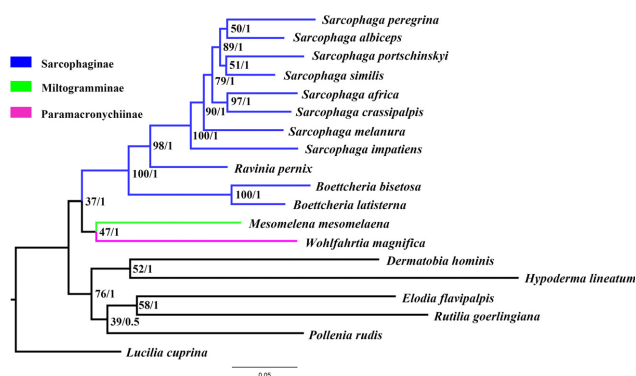




**Fig. 4.** Plot of pairwise Ka versus Ks of the mitochondrial genes of Sarcophagidae.

mitochondrial genes (Fig. 4), except *atp8*, for which only non-synonymous substitutions between *Sarcophaga africa* (locus: NC\_025944) (Fu et al., 2016) and *S. similis* (locus: NC\_025573) (Yan et al., 2016) were detected. The lower values of Ka than Ks indicate negative selection on sarcophagid mitochondrial genes. Specifically, the *cox1* gene has undergone strong negative selection, indicated by the extremely low Ka value compared to Ks value, which also implies the conservativeness of *cox1* gene.

ML and BI analyses give an identical result, with Sarcophagidae being monophyletic and Sarcophaginae as the sister group to (Paramacronychiinae + Miltogramminae) (Fig. 5). According to previous research the relationship of these three subfamilies is still unresolved. Pape (1996: 9) proposed a probable sister-group relationship between the Paramacronychiinae and Sarcophaginae, based on “the shared possession of a ventrally displaced acrophallus, lack of epiphallus and perhaps spherical female accessory glands”, which was corroborated by Giroux et al. (2010) in an explicit morphology-based phylogenetic analysis. This relationship was corroborated by Kutty et al. (2010) using



**Fig. 5.** Phylogeny of Sarcophagidae inferred from a mitochondrial dataset comprising 13 protein-coding genes and 2 rRNA genes. Numbers at nodes are bootstrap values (ML trees) / posterior probabilities (Bayesian trees).

a combination of mitochondrial and nuclear genes, although with low support. However, based on a large-scale (in terms of taxa) molecular phylogeny, Piwczyński et al. (2014) suggest a sister-group relationship of Sarcophaginae to (Paramacronychiinae + Miltogramminae), with Paramacronychiinae being paraphyletic, and Piwczyński et al.’s (2017) results are fully concordant with the above, with each subfamily being monophyletic.

Within the Sarcophaginae, *Boettcheria* is located as a basal branch, forming a sister group to (*Ravinia* + *Sarcophaga*). This is in conflict with morphology-based phylogenies of this subfamily, where *Ravinia* is subordinate to *Boettcheria* and *Sarcophaga* (Pape, 1994; Giroux et al., 2010). The monophyly of *Sarcophaga* is well supported (ML bootstrap = 100; BI posterior probability = 1), but the few taxa sampled and inclusion of only Old World species do not allow the testing of any of the biogeographic hypotheses proposed by Buenaventura et al. (2016) and Buenaventura & Pape (2017).

This study documents the first mitogenome of Miltogramminae, which contains 37 typical metazoan mitochondrial genes and retains the organization of the ancestral insect mitogenome. The subfamily relationship within Sarcophagidae is reconstructed for the first time as (Sarcophaginae (Paramacronychiinae, Miltogramminae)) based on mitogenomic data.

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## REFERENCES

- AZEREDO-ESPIN A.M.L., JUNQUEIRA A.C.M., LESSINGER A.C., LYRA M.L., TORRES T.T., DA SILVA F.R., MEIRELLES F.V. & MAIA A.A.M. 2004: *The complete mitochondrial genome of the human botfly Dermatobia hominis (Diptera: Oestridae). Unpublished poster, ESA Annual Meeting and Exhibition.* URL: [https://esa.confex.com/esa/2004/techprogram/paper\\_16801.htm](https://esa.confex.com/esa/2004/techprogram/paper_16801.htm)
- BÄNZIGER H. & PAPE T. 2004: Flowers, faeces and cadavers: natural feeding and laying habits of flesh flies in Thailand (Diptera: Sarcophagidae, *Sarcophaga* spp.). — *J. Nat. Hist.* **38**: 1681–1698.

- BUENAVENTURA E. & PAPE T. 2017: Multilocus and multiregional phylogeny reconstruction of the genus *Sarcophaga* (Diptera, Sarcophagidae). — *Mol. Phyl. Evol.* **107**: 619–629.
- BUENAVENTURA E., WHITMORE D. & PAPE T. 2016: Molecular phylogeny of the hyperdiverse genus *Sarcophaga* (Diptera: Sarcophagidae), and comparison between algorithms for identification of rogue taxa. — *Cladistics* **32**: 109–133.
- CAMERON S.L. 2014: Insect mitochondrial genomics: implications for evolution and phylogeny. — *Annu. Rev. Entomol.* **59**: 95–117.
- CERRETTI P., STIREMAN J.O., PAPE T., O'HARA J.E., MARINHO M.A.T., ROGNES K. & GRIMALDI D.A. 2017: First definitive fossil of an oestroid fly (Diptera: Calyptratae: Oestroidea) and the calibration of oestroid divergences. — *PLoS ONE* **12**(8): e0182101, 24 pp.
- FU X., CHE K., ZHU Z., LIU J. & GUO Y. 2016: The complete mitochondria genome of *Sarcophaga africa* (Diptera: Sarcophagidae). — *Mitochondr. DNA (A)* **27**: 2115–2116.
- GIROUX M., PAPE T. & WHEELER T.A. 2010: Towards a phylogeny of the flesh flies (Diptera: Sarcophagidae): morphology and phylogenetic implications of the acrophallus in the subfamily Sarcophaginae. — *Zool. J. Linn. Soc.* **158**: 740–778.
- GRANT J.R. & STOTHARD P. 2008: The CGView Server: a comparative genomics tool for circular genomes. — *Nucl. Acid. Res.* **36**: 181–184.
- GUO J., XIE K., CHE K., HU Z. & GUO Y. 2016: The complete mitochondria genome of *Ravinia pernix* (Diptera: Sarcophagidae). — *Mitochondr. DNA (A)* **27**: 2069–2070.
- HURST L. 2002: The Ka/Ks ratio: diagnosing the form of sequence evolution. — *Trends Genet.* **18**: 486–489.
- JUNQUEIRA A.C.M., AZEREDO-ESPIN A.M.L., PAULO D.F., MARINHO M.A.T., TOMSHO L.P., DRAUTZ-MOSES D.I., PURBOJATI R.W., RATAN A. & SCHUSTER S.C. 2016: Large-scale mitogenomics enables insights into Schizophora (Diptera) radiation and population diversity. — *Sci. Rep.* **6**: 21762, 13 pp.
- KATOH K. & STANDLEY D.M. 2016: A simple method to control over-alignment in the MAFFT multiple sequence alignment program. — *Bioinformatics* **32**: 1933–1942.
- KUTTY S.N., PAPE T., WIEGMANN B.M. & MEIER R. 2010: Molecular phylogeny of the Calyptratae (Diptera: Cyclorrhapha) with an emphasis on the superfamily Oestroidea and the position of Mystacinobiidae and McAlpine's fly. — *Syst. Entomol.* **35**: 614–635.
- KUTTY S.N., PONT A.C., MEIER R. & PAPE T. 2014: Complete tribal sampling reveals basal split in Muscidae (Diptera), confirms saprophagy as ancestral feeding mode, and reveals an evolutionary correlation between instar numbers and carnivory. — *Mol. Phylogenet. Evol.* **78**: 349–364.
- LALITHA S. 2000: Primer Premier 5.0. — *Biotech. Softw. Internet Rep.* **1**: 270–272.
- LANFEAR R., FRANDSEN P.B., WRIGHT A.M., SENFELD T. & CALCOTT B. 2016: PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. — *Mol. Biol. Evol.* **34**: 772–773.
- LIAO H., YANG X., LI Z., DING Y. & GUO Y. 2016: The complete mitochondria genome of *Parasarcophaga albiceps* (Diptera: Sarcophagidae). — *Mitochondr. DNA (A)* **27**: 4696–4698.
- LIBRADO P. & ROZAS J. 2009: DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. — *Bioinformatics* **25**: 1451–1452.
- LOEW H. 1848: Eine neue europäische Art der Gattung *Metopia*. — *Stettin. Entomol. Ztg.* **9**: 377–378.
- LOPES H.S. 1982: On *Eumacronychia sternalis* Allen (Diptera, Sarcophagidae) with larvae living on eggs and hatchlings [sic!] of the East Pacific green turtle. — *Rev. Bras. Biol.* **42**: 425–429.
- McKILLUP S.C., McKILLUP R.V. & PAPE T. 2000: Flies that are parasitoids of a marine snail: the larviposition behaviour and life cycles of *Sarcophaga megafilosia* and *Sarcophaga meiofilosia*. — *Hydrobiologia* **439**: 141–149.
- MEIER R., KWONG S., VAIDYA G. & NG P.K.L. 2006: DNA Barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. — *Syst. Biol.* **55**: 715–728.
- MEIKLEJOHN K.A., WALLMAN J.F., CAMERON S.L. & DOWTON M. 2012: Comprehensive evaluation of DNA barcoding for the molecular species identification of forensically important Australian Sarcophagidae (Diptera). — *Invertebr. Syst.* **26**: 515–525.
- MULLEN G.R., TRAUTH S.E. & SELLERS J.C. 1984: Association of a miltogrammine fly, *Eumacronychia nigricornis* Allen (Diptera: Sarcophagidae), with the brood burrows of *Sceloporus undulatus* (Latrielle) (Reptilia: Lacertillia). — *J. Georgian. Entomol. Soc.* **19**: 1–6.
- NELSON L.A., LAMBKIN C.L., BATTERHAM P., WALLMAN J.F., DOWTON M., WHITING M.F., YEATES D.K. & CAMERON S.L. 2012: Beyond barcoding: a mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). — *Gene* **511**: 131–142.
- PAPE T. 1994: The world *Blaesoxipha* Loew, 1861 (Diptera: Sarcophagidae). — *Entomol. Scand. (Suppl.)* **45**: 1–247.
- PAPE T. 1996: Catalogue of the Sarcophagidae of the world (Insecta: Diptera). — *Mem. Entomol. Int.* **8**: 1–558.
- PAPE T., BLAGODEROV V. & MOSTOVSKI M.B. 2011: Order Diptera Linnaeus, 1758. — *Zootaxa* **3148**: 222–229.
- PICKENS G. 1981: The life history and predatory efficiency of *Ravinia lherminieri* (Diptera: Sarcophagidae) on the face fly (Diptera: Muscidae). — *Can. Entomol.* **113**: 523–526.
- PIWCZYŃSKI M., SZPILA K. & PAPE T. 2014: A large-scale molecular phylogeny of flesh flies (Diptera: Sarcophagidae). — *Syst. Entomol.* **39**: 783–799.
- PIWCZYŃSKI M., PAPE T., SIKORA M., DEJA-SIKORA E., AKBARZADEH K. & SZPILA K. 2017: Molecular phylogeny of Miltogramminae (Diptera: Sarcophagidae): implications for classification, systematics and evolution of larval feeding strategies. — *Mol. Phyl. Evol.* **116**: 49–60.
- RAMAKODI M.P., SINGH B., WELLS J.D., GUERRERO F. & RAY D.A. 2015: A 454 sequencing approach to dipteran mitochondrial genome research. — *Genomics* **105**: 53–60.
- SHI J., GUO Y., ZHANG C., ZHOU Y., YAN J., LIAO H. & ZHA L. 2016: The complete mitochondrial genome of the flesh fly, *Parasarcophaga portschinskyi* (Diptera: Sarcophagidae). — *Mitochondr. DNA (A)* **27**: 1910–1911.
- SPOFFORD M.G., KURCZEWSKI F.E. & DOWNES JR W.L. 1989: Nearctic species of Miltogrammini (Diptera: Sarcophagidae) associated with species of Aculeata (Hymenoptera: Vespoidea, Pompiloidea, Sphecoidea, Apoidea). — *J. Kans. Entomol. Soc.* **62**: 254–267.
- STUCKY B.J. 2015: Infection behavior, life history, and host parasitism rates of *Emblemasoma erro* (Diptera: Sarcophagidae), an acoustically hunting parasitoid of the cicada *Tibicen dorsatus* (Hemiptera: Cicadidae). — *Zool. Stud.* **54**: 30, 17 pp.
- TRAUTH S.E. & MULLEN G.R. 1990: Additional observations on sarcophagid fly infestations of *Sceloporus undulatus* (Sauria: Iguanidae) egg clutches in Arkansas. — *Southwest. Nat.* **1**: 97–98.
- WEIGL S., TESTINE G., PARISI A., DANTAS-TORRES F., TRAVERSA D., COLWELL D.D. & OTRANTO D. 2010: The mitochondrial genome



- of the common cattle grub, *Hypoderma lineatum*. — *Med. Vet. Entomol.* **24**: 329–335.
- WIEGMANN B.M., TRAUTWEIN M.D., WINKLER I.S., BARR N.B., KIM J.W., LAMBKIN C., BERTONE M.A., CASSEL B.K., BAYLESS K.M., HEIMBERG A.M. ET AL. 2011: Episodic radiations in the fly tree of life. — *Proc. Natn. Acad. Sci. USA* **108**: 5690–5695.
- YAN J., LIAO H., ZHU Z., XIE K., GUO Y. & CAI J. 2016: The complete mitochondria genome of *Parasarcophaga similis* (Diptera: Sarcophagidae). — *Mitochondr. DNA (A)* **27**: 1642–1643.
- ZHANG N.X., ZHANG Y.J., YU G. & CHEN B. 2013: Structure characteristics of the mitochondrial genomes of Diptera and design and application of universal primers for their sequencing. — *Acta. Entomol. Sin.* **56**: 398–407 [in Chinese.]
- ZHANG C., FU X., ZHU Z., XIE K. & GUO Y. 2016a: The complete mitochondrial genome sequence of *Helicophagella melanura* (Diptera: Sarcophagidae). — *Mitochondr. DNA (A)* **27**: 3905–3906.
- ZHANG D., YAN L., ZHANG M., CHU H., CAO J., LI K., HU D. & PAPE T. 2016b: Phylogenetic inference of calyptrates, with the first mitogenomes for Gasterophilinae (Diptera: Oestridae) and Paramacronychiinae (Diptera: Sarcophagidae). — *Int. J. Biol. Sci.* **5**: 489–504.
- ZHAO Z., SU T., CHESTERS D., WANG S., HO S.Y.W., ZHU C., CHEN X. & ZHANG C. 2013: The mitochondrial genome of *Elodia flavipalpis* Aldrich (Diptera: Tachinidae) and the evolutionary timescale of tachinid flies. — *PLoS ONE* **8**: e61814, 15 pp.
- ZHONG M., WANG X., LIU Q., WU C. & WEN J. 2016: The complete mitochondrial genome of the flesh fly, *Boettcherisca peregrina* (Diptera: Sarcophagidae). — *Mitochondr. DNA (A)* **27**: 106–108.

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